

WHAT IS CLAIMED IS:

1 1. A zinc finger protein that binds to a target site, wherein the target site
2 has a nucleotide sequence as specified in Table 3 or 4.

1 2. The zinc finger protein of claim 1, comprising at least one finger of the
2 C2H2 class of zinc fingers.

1 3. The zinc finger protein according to claim 2, wherein the target site is
2 one of the nucleotide sequences in a row of Table 3 or 4 and positions -1 to +6 in at least one
3 of the zinc fingers are occupied by a segment of seven contiguous amino acids as specified in
4 the row.

1 4. The zinc finger protein according to claim 3, wherein positions -1 to
2 +6 in each of the three zinc fingers are occupied by first, second and third segments of seven
3 contiguous amino acids as specified in a row of Table 3.

1 5. The zinc finger protein according to claim 4, wherein the segments
2 have the amino acid sequences specified for one of the zinc finger proteins listed in Table 3,
3 wherein the zinc finger protein is selected from the group consisting of BVO 13A, EP10A,
4 GATA82Z7678, HBV 3, HP38 4A, HUM 17A, HUM 19A, MTS 5A, MX1E, PDF 5A, RAT
5 24A, SAN 16A, USX 3A, VEGF 1, VEGF 1*3, VEGF 1A, VEGF 1B, VEGF 1C, VEGF 1D,
6 VG 10A, VG 1B, VG 4A, VG 8A, VOP 28A-2, VOP 30A-4, VOP 32A-6, VOP 32B-7, VOP
7 35A-10, ZEN-7A 1, VOP 29A-3, VOP 32C, VOP 32D, VOP 32E, VOP 32F, VOP 32G,
8 VOP 32H, VOP 32I and VOP 32J.

1 6. The zinc finger protein according to claim 2, wherein the zinc finger
2 protein comprises six zinc fingers, and positions -1 to +6 in at least one of the six zinc fingers
3 is occupied by a segment of seven contiguous amino acids as specified in Table 4.

1 7. The zinc finger protein according to claim 2, wherein the zinc finger
2 protein comprises six zinc fingers, and positions -1 to +6 in each of the six zinc fingers are
3 occupied by a segment of seven contiguous amino acids as specified in a row of Table 4.

1 8. The zinc finger protein according to claim 7, wherein the segments
2 have the amino acid sequences specified for a zinc finger protein selected from the group
3 consisting of BVO 10A-9A, BVO 12A-11B and BVO 14B-13A as listed in Table 4.

1 9. The zinc finger protein according to claim 1, wherein the zinc finger
2 protein is a fusion protein comprising a regulatory domain.

1 10. The zinc finger protein according to claim 9, wherein the fusion
2 protein comprises a plurality of regulatory domains.

1 11. The zinc finger protein according to claim 9, wherein the regulatory
2 domain is an activation domain.

1 12. The zinc finger protein according to claim 11, wherein the activation
2 domain is selected from the group consisting of (a) VP16, (b) p65, and (c) functional
3 fragments of (a) and (b).

1 13. The zinc finger protein according to claim 9, wherein the regulatory
2 domain is a repressor domain.

1 14. The zinc finger protein according to claim 13, wherein the repressor
2 domain is selected from the group consisting of (a) KRAB, (b) methyl binding domain
3 protein 2B, (c) v-ErbA repressor domain, and (d) functional fragments of (a), (b) and (c).

1 15. A zinc finger protein that binds to a target site having a nucleotide
2 sequence as specified in Table 3 or 4 whereby the zinc finger protein can modulate
3 angiogenesis when introduced into an animal having a genome comprising a VEGF gene
4 comprising the target site.

1 16. The zinc finger protein of claim 15, comprising at least three fingers of
2 the C₂H₂ class of zinc fingers.

1 17. The zinc finger protein according to claim 16, wherein the target site is
2 one of the nucleotide sequences in a row of Table 3 or 4 and positions -1 to +6 in at least one
3 of the zinc fingers are occupied by a segment of seven contiguous amino acids as specified in
4 the row.

1 18. The zinc finger protein according to claim 17, wherein positions -1 to
2 +6 in each of the three zinc fingers are occupied by first, second and third segments of seven
3 contiguous amino acids as specified in a row of Table 3.

1 19. The zinc finger protein according to claim 16, wherein the zinc finger
2 protein comprises six zinc fingers, and positions -1 to +6 in at least one of the six zinc fingers
3 is occupied by a segment of seven contiguous amino acids as specified in Table 4.

1 20. The zinc finger protein according to claim 19, wherein the zinc finger
2 protein comprises six zinc fingers, and positions -1 to +6 in each of the six zinc fingers are
3 occupied by a segment of seven contiguous amino acids as specified in a row of Table 4.

1 21. A nucleic acid encoding a polypeptide, wherein the polypeptide
2 comprises a zinc finger according to claim 1.

1 22. A nucleic acid encoding a polypeptide, wherein the polypeptide
2 comprises a zinc finger protein according to claim 4.

1 23. A nucleic acid encoding a polypeptide, wherein the polypeptide
2 comprises a zinc finger protein according to claim 7.

1 24. A nucleic acid encoding a polypeptide, wherein the polypeptide
2 comprises a zinc finger protein according to claim 9.

1 25. A method for modulating expression of a VEGF gene, the method
2 comprising contacting a target site of a nucleic acid within a cell with a zinc finger protein,
3 wherein the target site has a nucleotide sequence as specified in Table 3 or 4 and binding of
4 the zinc finger protein to the target site modulates expression of the VEGF gene in the cell.

1 26. The method according claim 25, wherein the expression of a plurality
2 of splice variants of the VEGF gene is modulated.

1 27. The method according to claim 25, wherein a plurality of target sites
2 are contacted with a plurality of zinc finger proteins and each zinc finger protein binds to a
3 distinct target site.

1 28. The method according to claim 27, wherein each of the plurality of
2 zinc finger proteins is a fusion protein.

1 29. The method according to claim 28, wherein each of the zinc finger
2 proteins is a fusion protein comprising a regulatory domain.

1 30. The method according to claim 29, wherein each zinc finger protein is
2 fused to a different regulatory domain.

1 31. The method according to claim 25, wherein the zinc finger protein
2 comprises at least three fingers of the C₂H₂ class of zinc fingers.

1 32. The method according to claim 31, wherein positions -1 to +6 in each
2 of the three zinc fingers are occupied by first, second and third segments of seven contiguous
3 amino acids as specified in a row of Table 3.

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1 33. The method according to claim 31, wherein the zinc finger protein
2 comprises six zinc fingers, and positions -1 to +6 in each of the six zinc fingers are occupied
3 by a segment of seven contiguous amino acids as specified in a row of Table 4.

1 34. The method according to claim 25, wherein the zinc finger protein is a
2 fusion protein comprising a regulatory domain.

1 35. The method according to claim 34, wherein the method further
2 comprises administering the zinc finger protein in combination with a delivery vehicle.

1 36. The method according to claim 34, wherein the method further
2 comprises administering a nucleic acid encoding the zinc finger protein into the cell.

1 37. The method according to claim 36, wherein administering comprises
2 delivering the nucleic acid into the cell in a naked form.

1 38. The method according to claim 36, wherein the nucleic acid is
2 contained within an expression vector and is operably linked to a promoter, and administering
3 comprises delivering the vector into the cell.

1 39. The method according to claim 38, wherein the expression vector is a
2 viral expression vector.

1 40. The method according to claim 39, wherein the expression vector is
2 selected from the group consisting of a retroviral expression vector, an adenoviral expression
3 vector, and an AAV expression vector.

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1 41. The method according to claim 38, wherein the promoter is an
2 inducible promoter.

1 42. The method according to claim 34, wherein regulatory domain
2 comprises an activation domain and binding of the zinc finger protein to the target site
3 activates transcription of the VEGF gene in the cell.

1 43. The method according to claim 42, wherein the cell is a population of
2 cells.

1 44. The method according to claim 43, wherein activation of VEGF
2 transcription activates angiogenesis in the population of cells.

1 45. The method according to claim 44, wherein the population of cells is a
2 cell culture.

1 46. The method according to claim 44, wherein the population of cells are
2 in a mammalian subject.

1 47. The method according to claim 36, wherein the zinc finger protein or
2 zinc finger protein nucleic acid are administered in an amount effective to treat a disease or
3 injury.

1 48. ^{SP} The method according to claim 47, wherein the disease or injury is
2 selected from the group consisting of atherosclerosis, ischemia and arthritis.

1 49. The method according to claim 47, wherein the subject has a wound
2 and the amount administered is effective to treat the wound.

1 50. The method according to claim 47, wherein the subject has an ulcer
2 and the amount administered is effective to treat the ulcer.

1 51. The method according to claim 42, wherein activation of VEGF
2 transcription activates lymphogenesis in the population of cells.

1 52. The method according to claim 42, wherein activation of VEGF
2 transcription activates myelopoiesis in the population of cells.

1 53. The method according to claim 42, wherein the activation domain is
2 selected from the group consisting of (a) VP16, (b) p65, (c) functional fragments of (a) and
3 (b).

1 54. The method according to claim 34, wherein the regulatory domain is a
2 repressor domain and binding of the zinc finger protein to the target site represses
3 transcription of the VEGF gene in the cell.

1 55. The method according to claim 54, wherein the cell is a population of
2 cells.

1 56. The method according to claim 55, wherein repression of VEGF
2 transcription represses angiogenesis in the population of cells.

1 57. The method according to claim 55, wherein the population of cells is a
2 cell culture.

1 58. The method according to claim 55, wherein the population of cells are
2 in a mammalian subject.

1 59. The method according to claim 58, wherein the zinc finger protein or
2 zinc finger protein nucleic acid are administered in an amount effective to treat a disease or
3 injury.

1 60. The method according to claim 59, wherein the disease is a tumor.

1 61. The method according to claim 54, wherein the repressor domain is
2 selected from the group consisting (a) KRAB, (b) methyl binding domain protein 2B, (c) v-
3 ErbA repressor domain, and (d) functional fragments of (a), (b) and (c).

1 62. The method according to claim 25, wherein the target site is located in
2 a single type of VEGF gene, and binding of the zinc finger protein to the target site modulates
3 expression of the single VEGF gene in the cell.

1 63. The method according to claim 25, wherein the target site is located in
2 a plurality of different types of VEGF genes, and binding of the zinc finger protein to the
3 target site modulates expression of the plurality of VEGF genes.

1 64. The method according to claim 63, wherein the target site comprises a
2 nucleotide sequence bound by a protein selected from the group consisting of EP10A,
3 GATA82Z678, HBV 3, HP38 4A, HUM 17A, MTS 5A, PDF 5A, USX 3A, VEGF 1,
4 VEGF1*3, VEGF 1A, VG 10A, VG 1B, VG 4A, VG8A, VOP28A-2, VOP 30A-4, and ZEN-
5 7A 1.

1 65. The method according to claim 64, wherein the target site is the
2 nucleotide sequence recognized by VOP 28A-2.

1 66. The method of according to claim 64, wherein the target site is the
2 nucleotide sequence recognized by VOP 30A-4.

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1 67. A method for modulating angiogenesis comprising introducing a zinc
2 finger protein into an animal having a genome comprising a target site within a VEGF gene,
3 whereby the zinc finger protein binds to the target site and thereby modulates angiogenesis in
4 the animal.

1 68. The method according to claim 67, wherein the modulation of
2 angiogenesis comprises inhibition of new blood vessel formation.

1 69. The method according to claim 67, wherein modulation of
2 angiogenesis comprises stimulation of new blood vessel formation.

1 70. The method according to claim 69, wherein the blood vessels are
2 nonhyperpermeable.

1 71. The method according to claim 67, wherein the zinc finger protein
2 binds to a target site specified in Table 3 or 4.

1 72. The method according to claim 71, wherein positions -1 to +6 in each
2 of three zinc fingers are occupied by first, second and third segments of seven contiguous
3 amino acids as specified in a row of Table 3.

1 73. The method according to claim 71, wherein the zinc finger protein
2 comprises six zinc fingers, and positions -1 to +6 in each of the six zinc fingers are occupied
3 by a segment of seven contiguous amino acids as specified in a row of Table 4.

1 74. The method according to claim 67, wherein the target site is present in
2 a plurality of VEGF genes, whereby the zinc finger protein binds to the target site in the
3 plurality of genes, thereby modulating expression of the plurality of VEGF genes.

1 75. The method according to claim 67, wherein introducing comprises
2 introducing a plurality of zinc finger proteins into the animal, each zinc finger protein binding
3 to a different target site in the same gene.

1 76. The method according to claim 75, wherein each of the zinc finger
2 proteins is a fusion protein comprising a regulatory domain.

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1 77. The method according to claim 76, wherein each zinc finger protein is
2 fused to a different regulatory domain.

1 78. A method of treating ischemia, comprising administering a zinc finger
2 protein that binds to a target site specified in Table 3 or 4 into an animal having ischemia,
3 wherein the zinc finger protein is administered in an amount effective to treat ischemia.

1 79. The method of claim 78, wherein the animal has a genome comprising
2 a VEGF gene comprising the target site and the zinc finger protein binds to the target site.

1 80. The method according to claim 79, wherein the zinc finger protein
2 comprises at least three fingers of the C₂H₂ class of zinc fingers.

1 81. The method according to claim 80, wherein positions -1 to +6 in each
2 of the three zinc fingers are occupied by first, second and third segments of seven contiguous
3 amino acids as specified in a row of Table 3.

1 82. The method according to claim 80, wherein the zinc finger protein
2 comprises six zinc fingers, and positions -1 to +6 in each of the six zinc fingers are occupied
3 by a segment of seven contiguous amino acids as specified in a row of Table 4.

1 83. A method for screening for a modulator of expression of a VEGF gene,
2 the method comprising:

3 (a) contacting a test cell with a zinc finger protein and a test agent,
4 wherein the zinc finger protein comprises at least one zinc finger that binds to a target site,
5 the target site having a nucleotide sequence as specified in Table 3 or 4;

6 (b) comparing the level of expression of the VEGF gene in the test cell
7 with a baseline level, a statistically significant difference in the level of expression in the test
8 cell relative to the baseline level indicating that the test agent is a potential modulator of
9 VEGF gene expression.

1 84. The method of claim 83, wherein the zinc finger is a fusion protein
2 comprising an activation domain, and a lower level of expression in the test cell relative to
3 the baseline level indicates that the test agent is a repressor of the VEGF gene.

1 85. The method of claim 83, wherein the zinc finger protein is a fusion
2 protein comprising a repressor domain, and an increased level of expression in the test cell
3 relative to the baseline level indicates that the test agent is an activator of the VEGF gene.

1 86. A pharmaceutical composition comprising a nucleic acid according to
2 claim 14 operably linked to a regulatory sequence and a pharmaceutically acceptable carrier
3 or diluent, wherein the regulatory sequence allows for expression of the nucleic acid in a cell.

1 87. The pharmaceutical composition according to claim 86, wherein the
2 nucleic acid is contained in an expression vector.

1 88. The pharmaceutical composition according to claim 87, wherein the
2 expression vector is a viral expression vector.

1 89. The pharmaceutical composition according to claim 88, wherein the
2 expression vector is selected from the group consisting of a retroviral expression vector, an
3 adenoviral expression vector, and an AAV expression vector.

1 90. A pharmaceutical composition comprising a zinc finger protein
2 according to claim 1 and a pharmaceutically acceptable carrier or diluent.

1 91. A zinc finger protein comprising a plurality of zinc fingers, wherein at
2 least one of the plurality of zinc fingers is occupied by a segment of seven contiguous amino
3 acids as specified in a row of Table 3 or 4.

1 92. The zinc finger protein of claim 91, wherein the zinc finger protein is a
2 three finger zinc finger protein and the at least one zinc finger is occupied by a segment of
3 seven contiguous amino acids as specified in a row of Table 3.

1 93. The zinc finger protein of claim 92, wherein at least two of the zinc
2 fingers are occupied by a segment of seven contiguous amino acids as specified in a row of
3 Table 3.

1 94. The zinc finger protein of claim 93, wherein all three of the zinc
2 fingers are occupied by a segment of seven contiguous amino acids as specified in a row of
3 Table 3.

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1 95. The zinc finger protein of claim 91, wherein the zinc finger protein is a
2 six finger zinc finger protein and the at least one zinc finger is occupied by a segment of
3 seven contiguous amino acids as specified in a row of Table 4.

1 96. The zinc finger protein of claim 95, wherein at least three of the zinc
2 fingers are occupied by a segment of seven contiguous amino acids as specified in a row of
3 Table 4.

1 97. ~~The zinc finger protein of claim 96, wherein all six of the zinc fingers~~
2 ~~are occupied by a segment of seven contiguous amino acids as specified in a row of Table 4.~~

1 98. A method for treating a wound comprising introducing a zinc finger
2 protein into an animal having a genome comprising a target site within a VEGF gene,
3 whereby the zinc finger protein binds to the target site, such binding accelerating healing of
4 the wound.